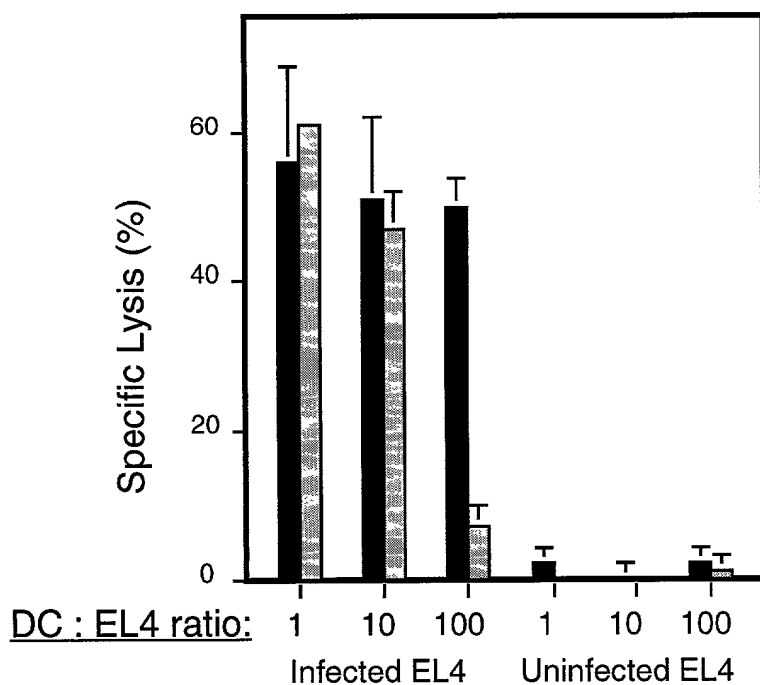
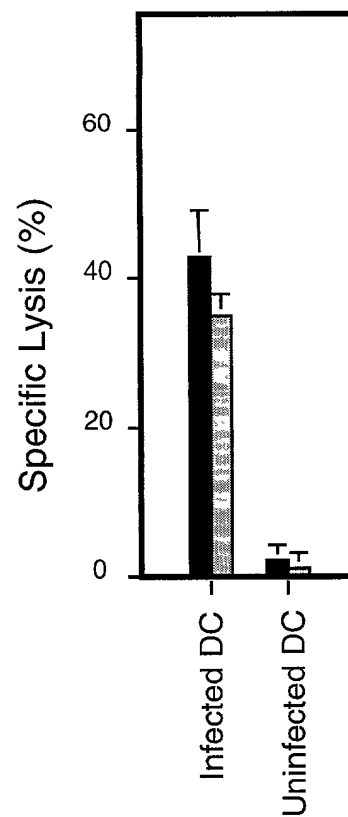


Figure 1B / A-D
Albert et al.

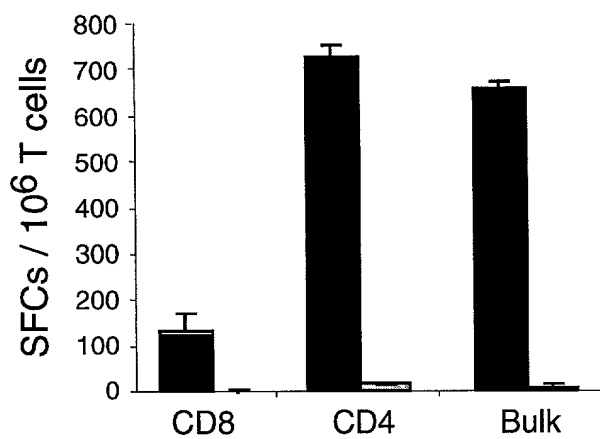
a



b



c



d

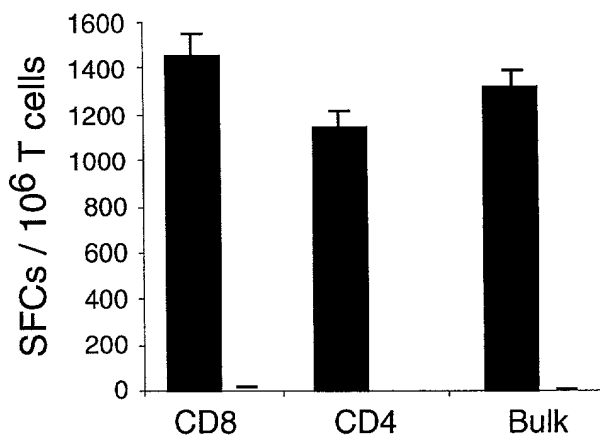
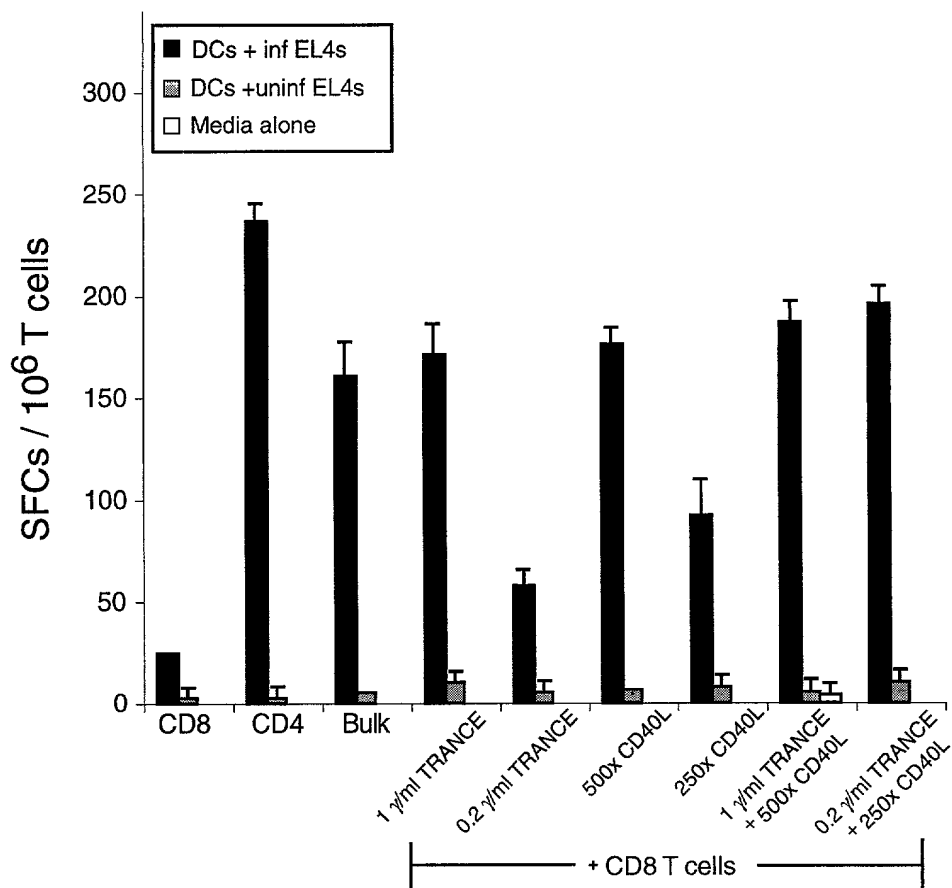


Figure 262 A-B
Albert et al.

a



b

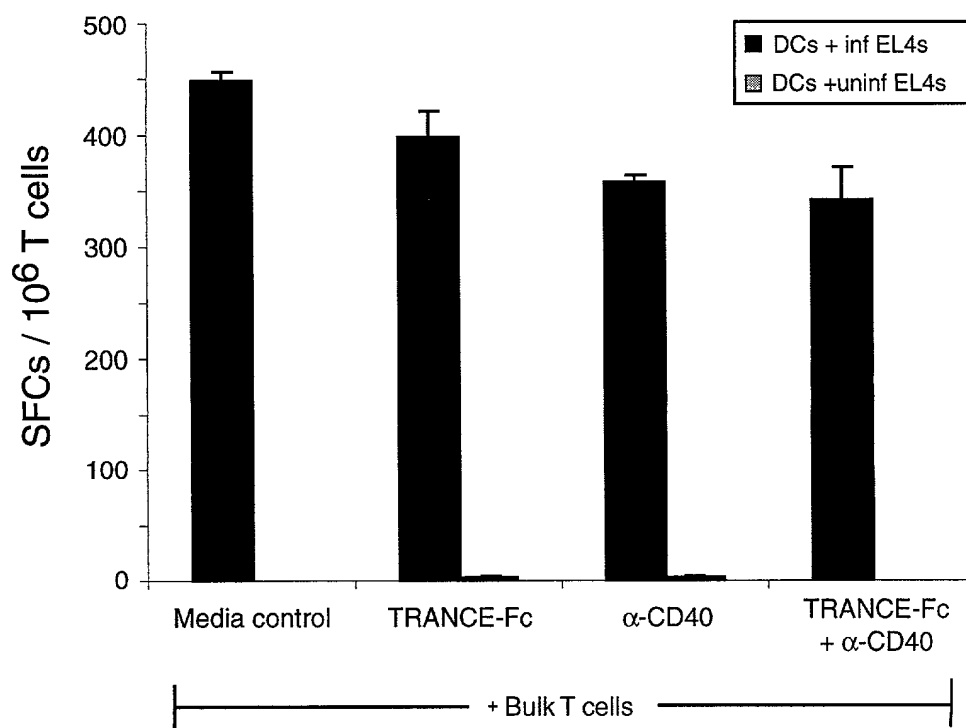
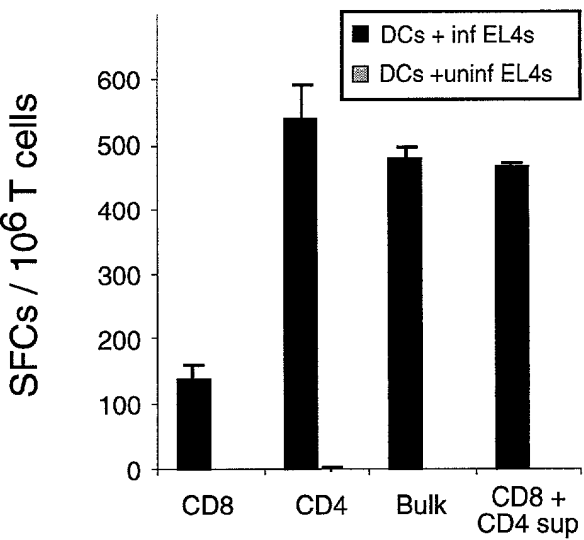
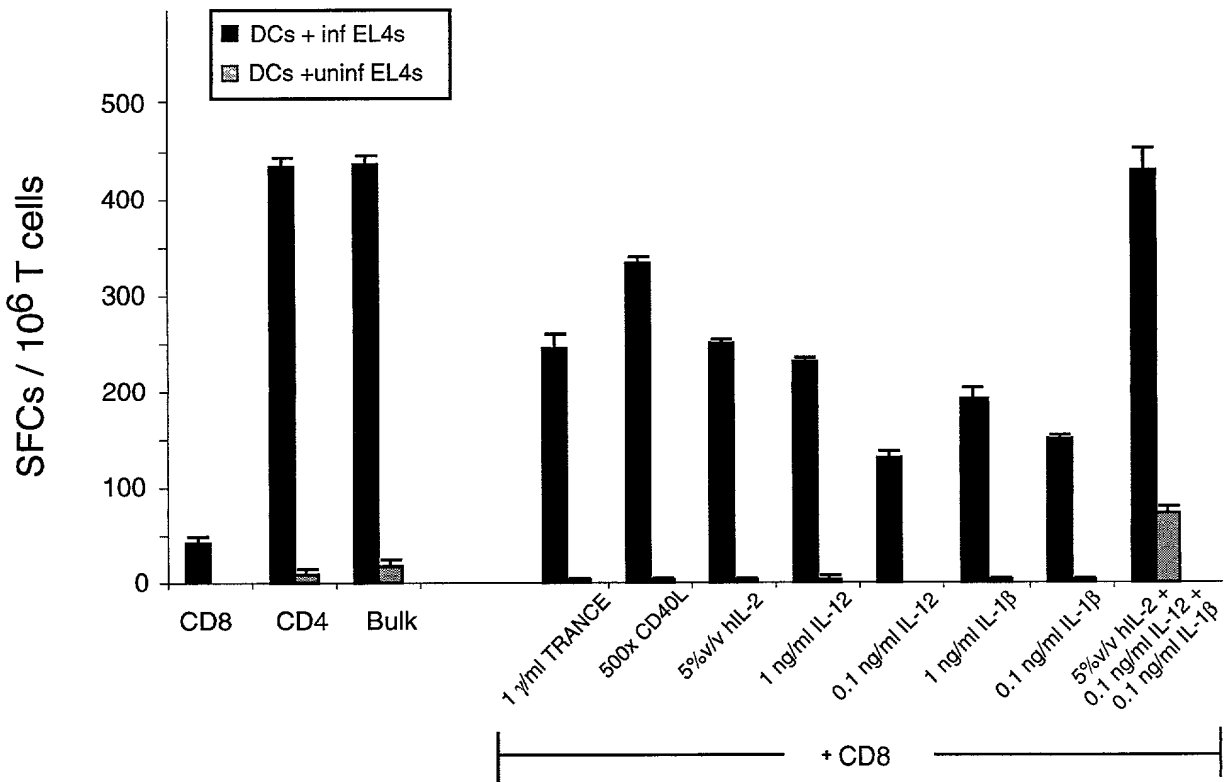


Figure 3/3 A-D
Albert et al.

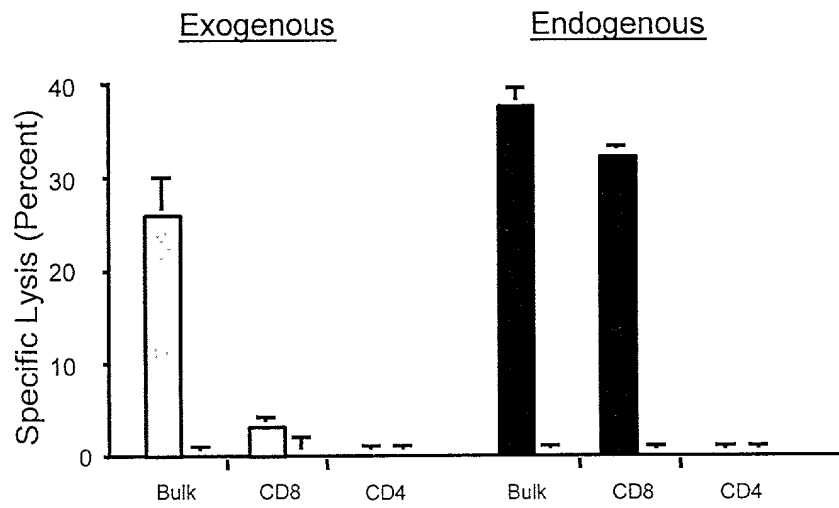
a



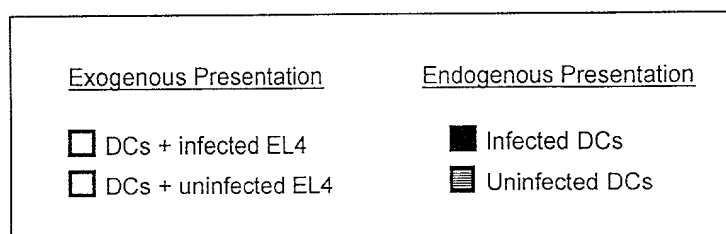
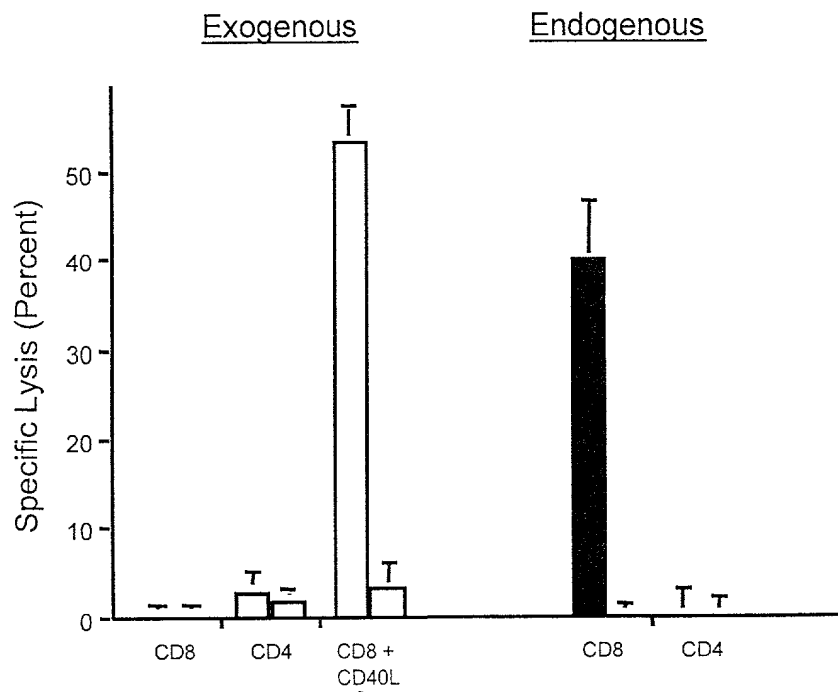
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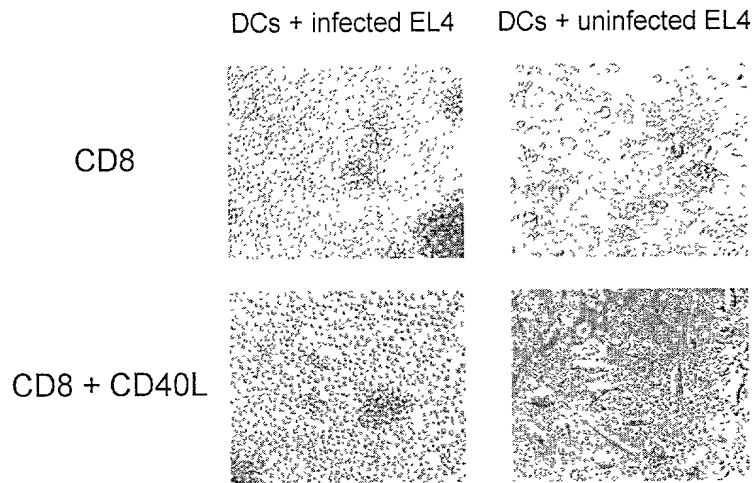
A



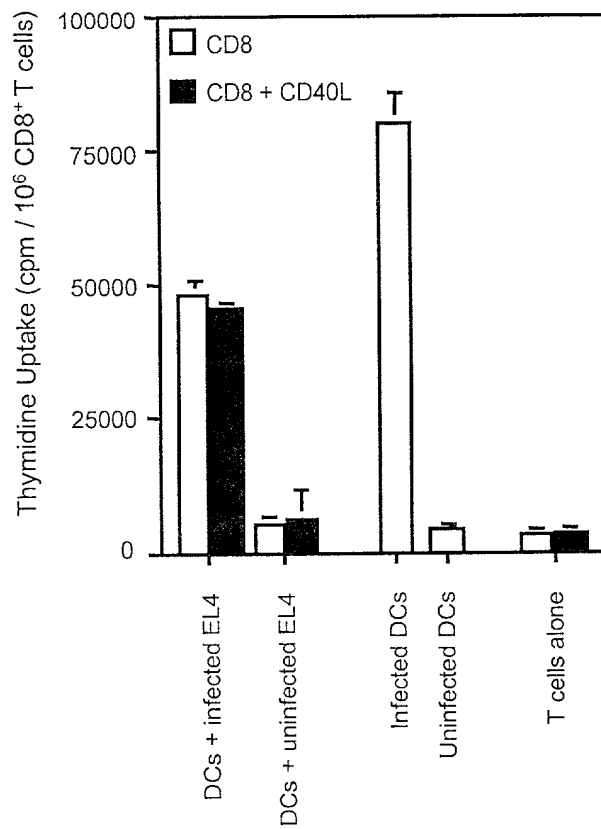
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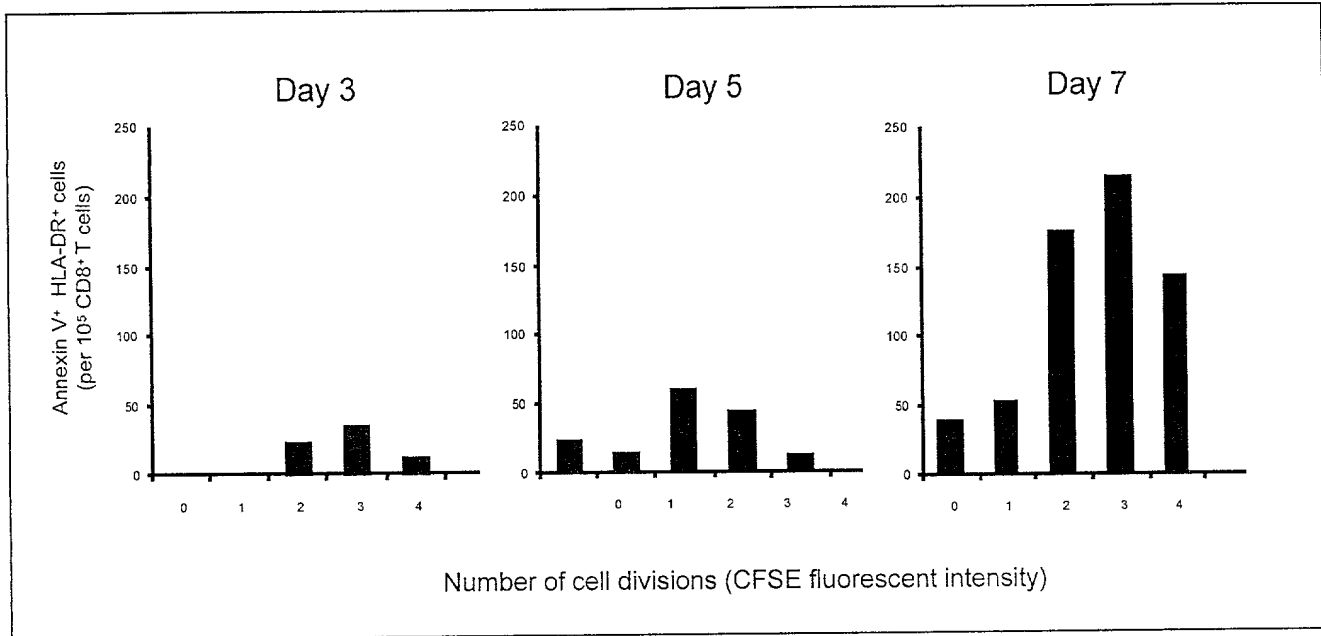
A



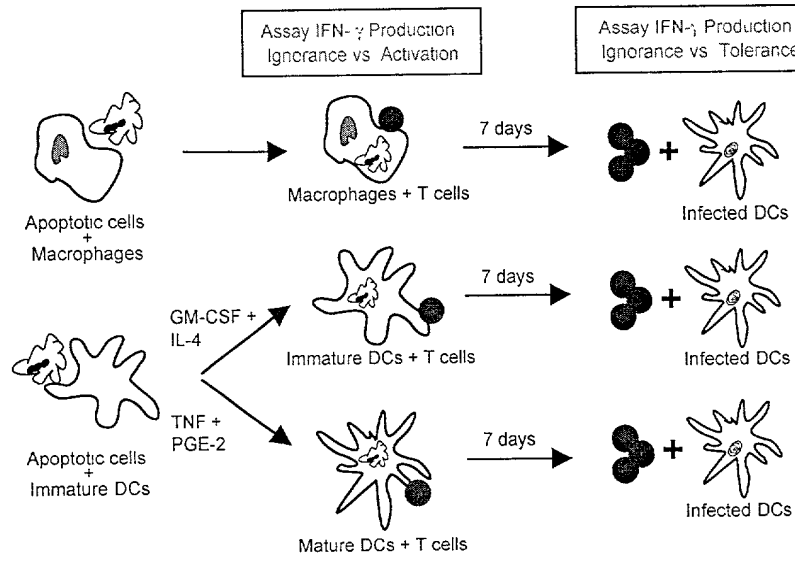
B



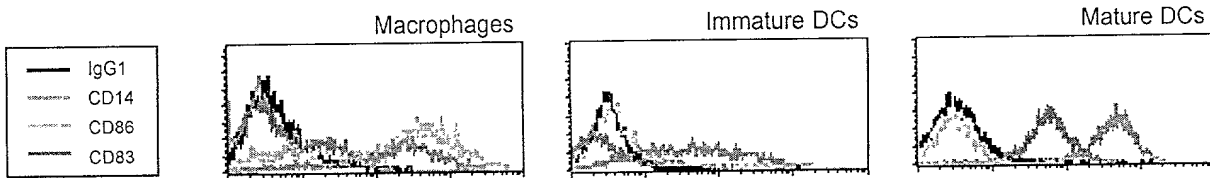
6
Figure 3, Albert et al.



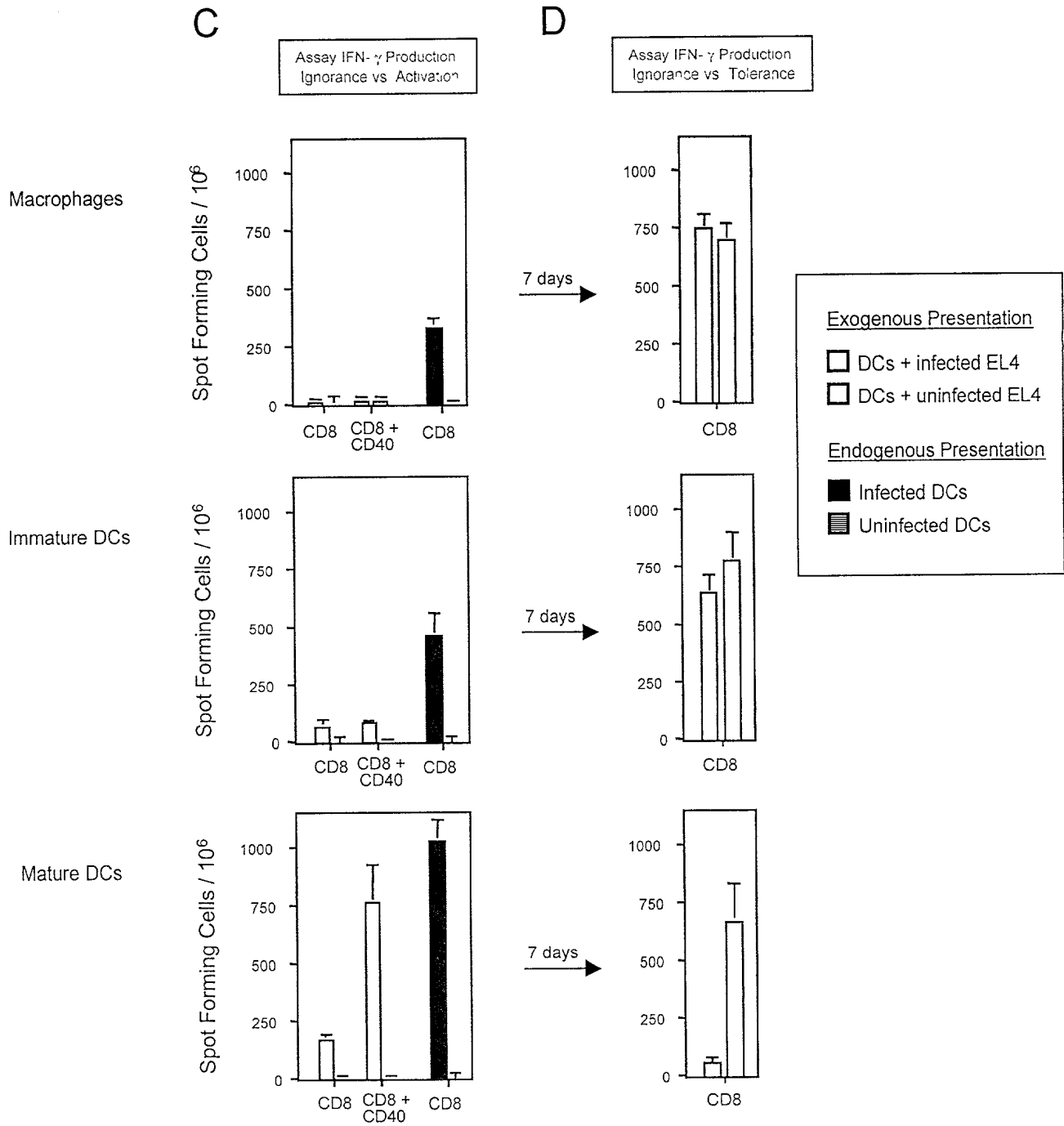
A



B



7
Figure 4, Albert et al.



E

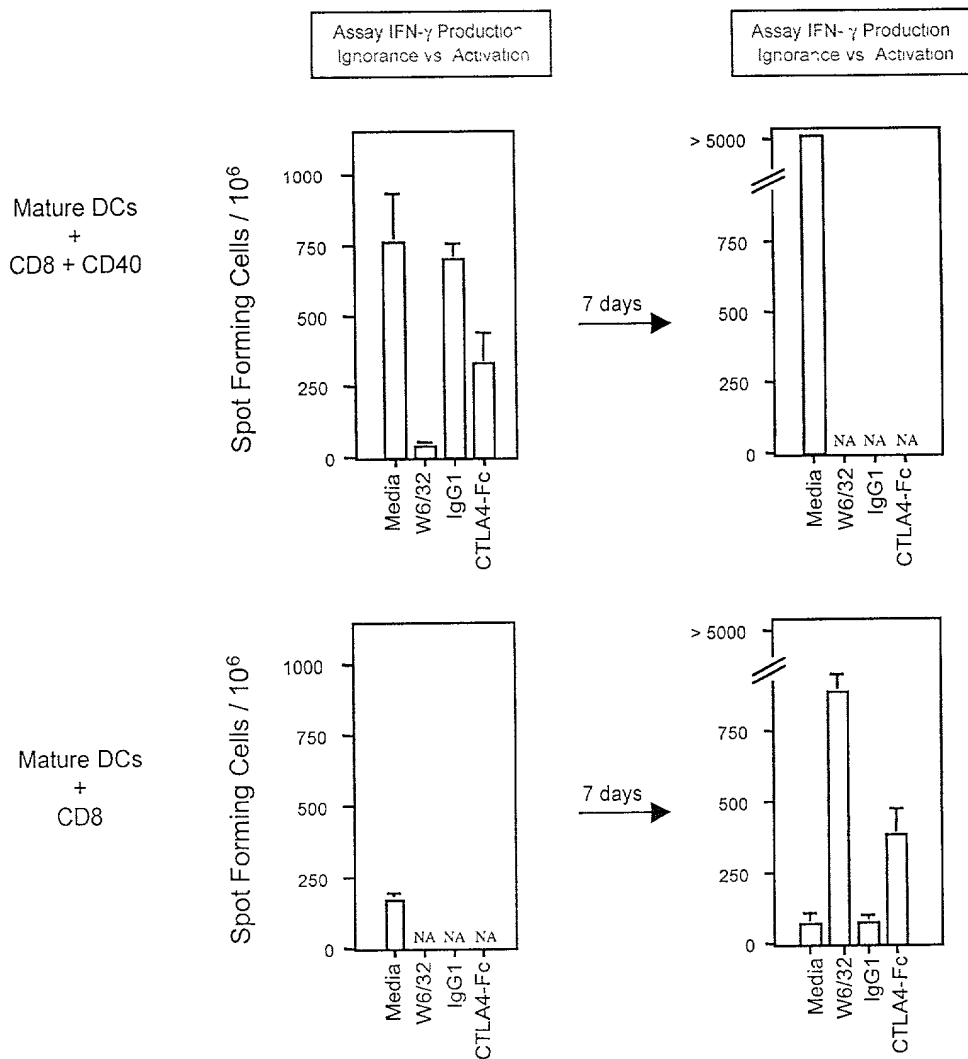
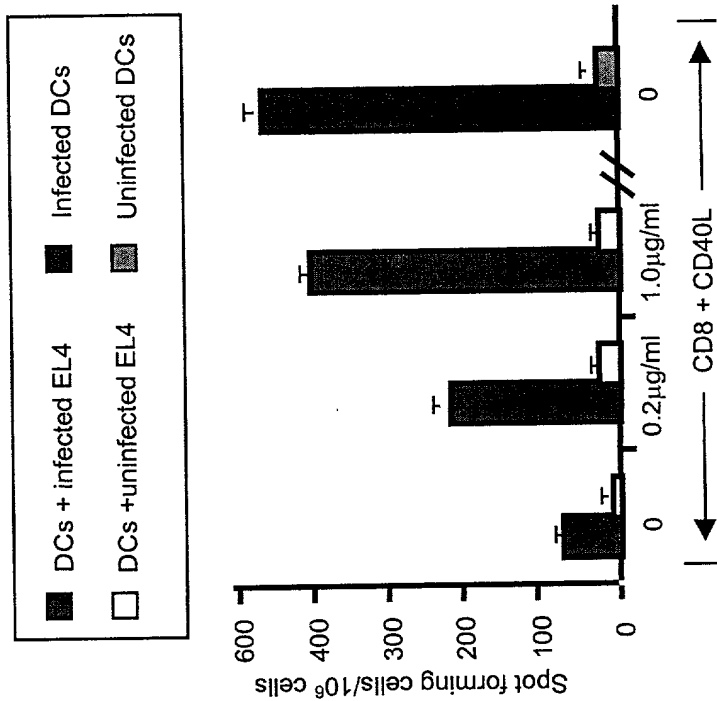
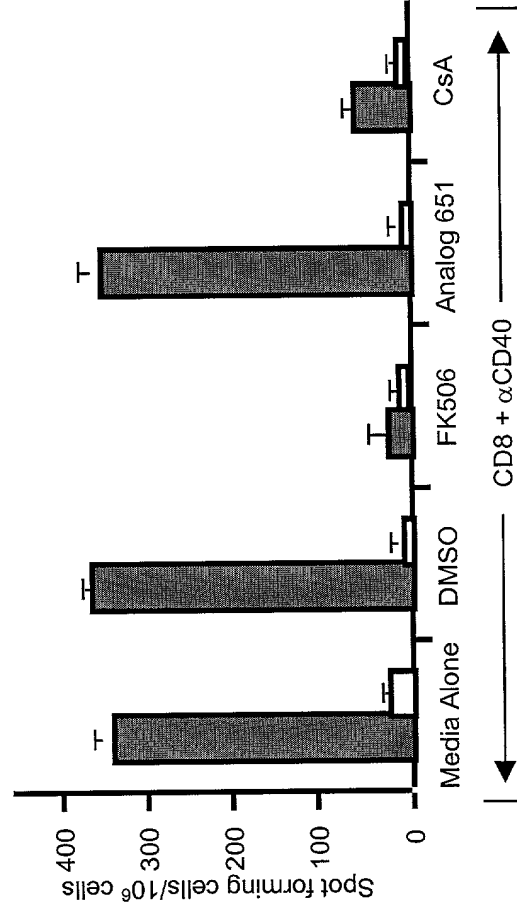
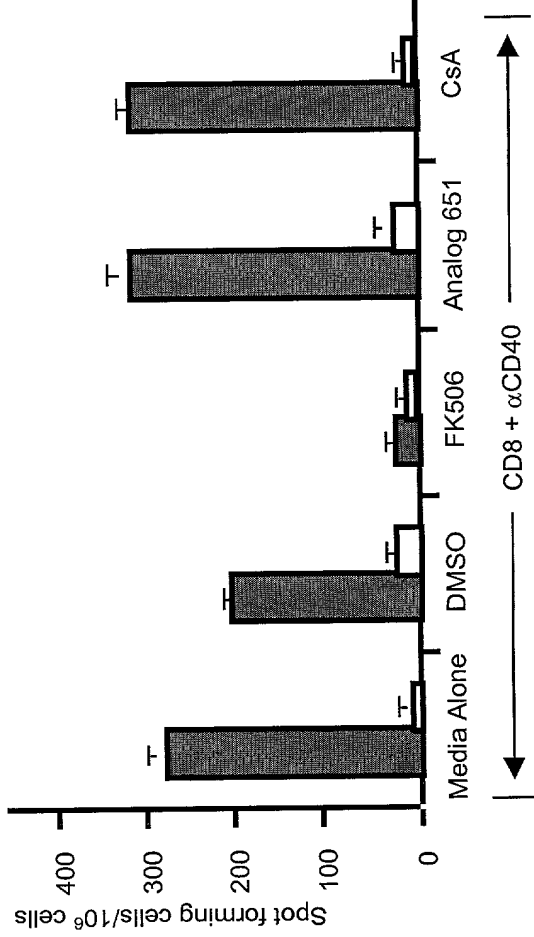
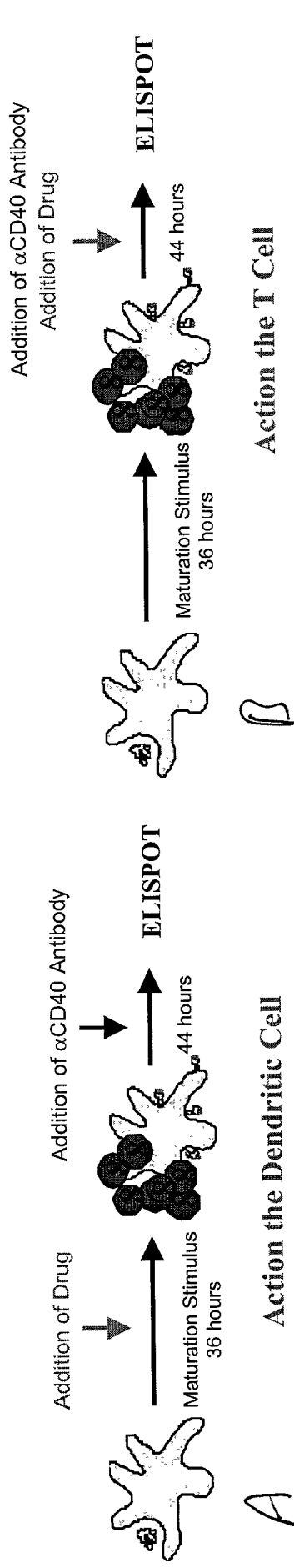


Fig. 8

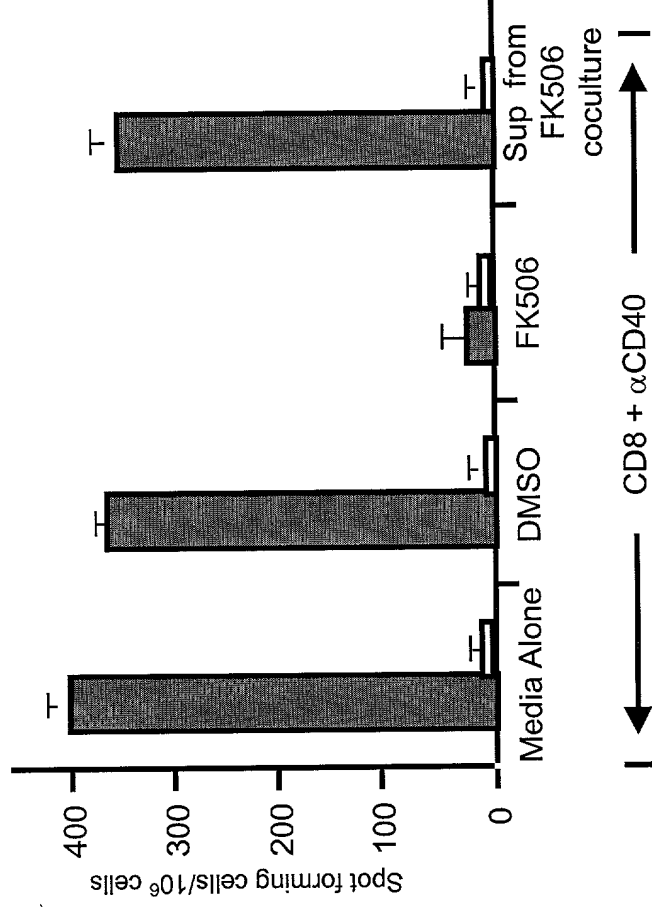
CD4 helper cells 'license' the DC to cross-prime via CD40 ligation



FK506, but not CsA inhibits cross-priming by affecting the DC



No residual FK506 remains in coculture



Cocultures were established as previously described with the addition of FK506 during the 36 hour DC-Apoptotic cell coculture. DCs were collected, washed, counted and plated in wells containing purified CD8+ T cells with α CD40 antibody with the addition of supernatant from the FK506 DC-Apoptotic cell coculture to untreated DCs. No residual FK506 remained in the coculture to inhibit T cell activation. Red bars, DCs + infected EL4 cells; White bars, DCs + uninfected EL4 cells.

FK506 selectively affects the exogenous MHC I pathway

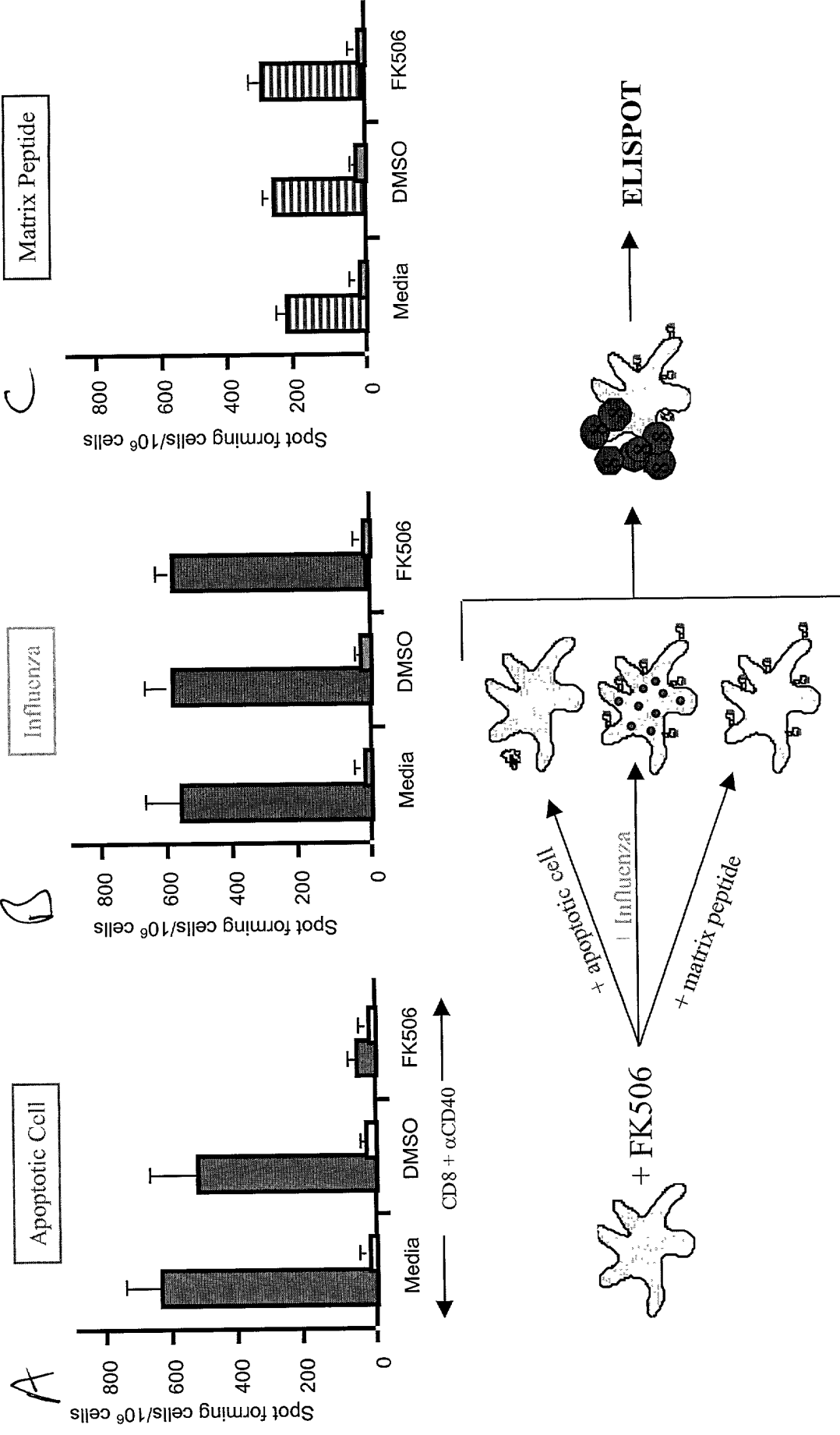
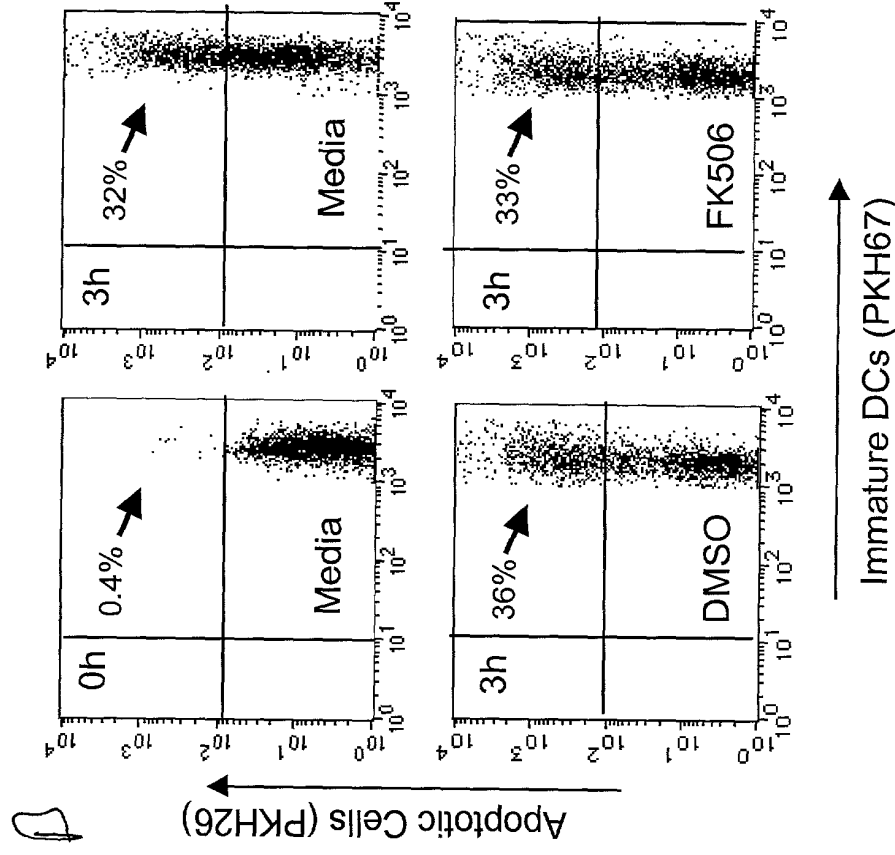
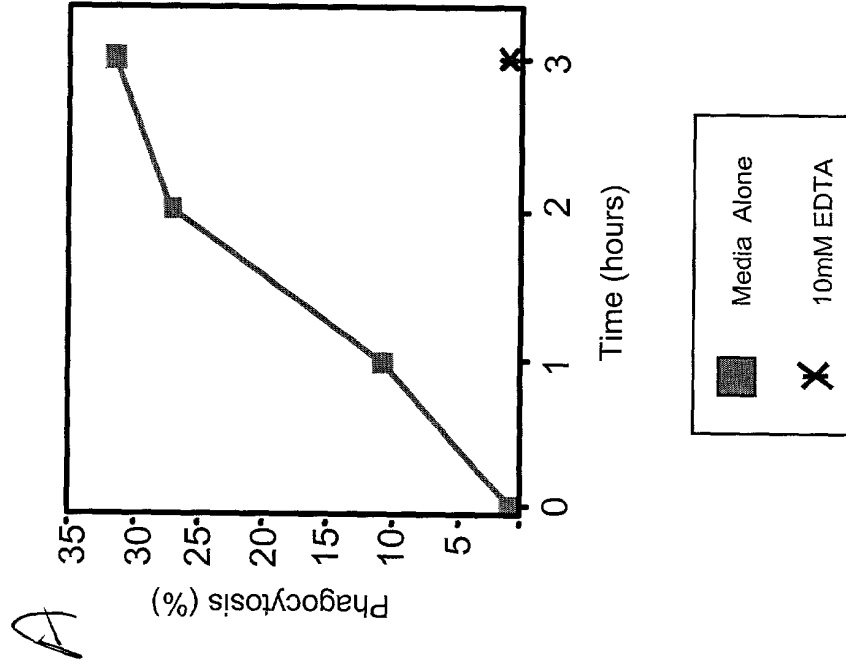


FIG 11 A-β

FK506 does not inhibit phagocytosis

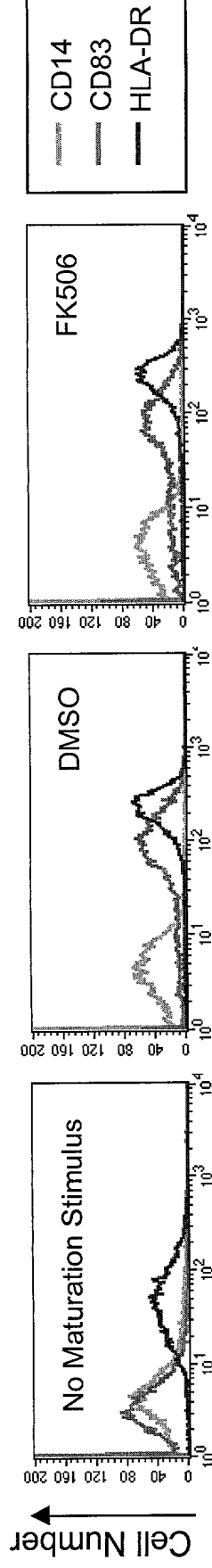


EL4 cells were dyed with PKH26, UVB irradiated and allowed to undergo apoptosis for 8 hours. Day 6 immature DCs were treated with 0.5μM FK506 for 24 hours, dyed with PKH67 and then cocultured with the apoptotic cells. Cocultures were then analyzed by FACS, gating on dendritic cells. Double positive cells were scored as a measure of percent phagocytosis. FK506 does not inhibit antigen capture.

FIG 11C

Cell Number

FK506 does not inhibit Dendritic Cell maturation



Cultures were established as previously described with the addition of 0.5 μ M FK506 during the 36 hour DC-Apoptotic cell coculture. DCs were collected, washed and stained for HLA-DR. HLA-DR⁺ DCs were then gated on to exclude apoptotic debris and analyzed by FACS for their CD14, CD83 and HLA-DR expression. FK506 does not act to inhibit cross-priming by affecting DC maturation.

Fig 11D

Cellular Interactions

FK506 does not inhibit generation of MHC I / peptide complex

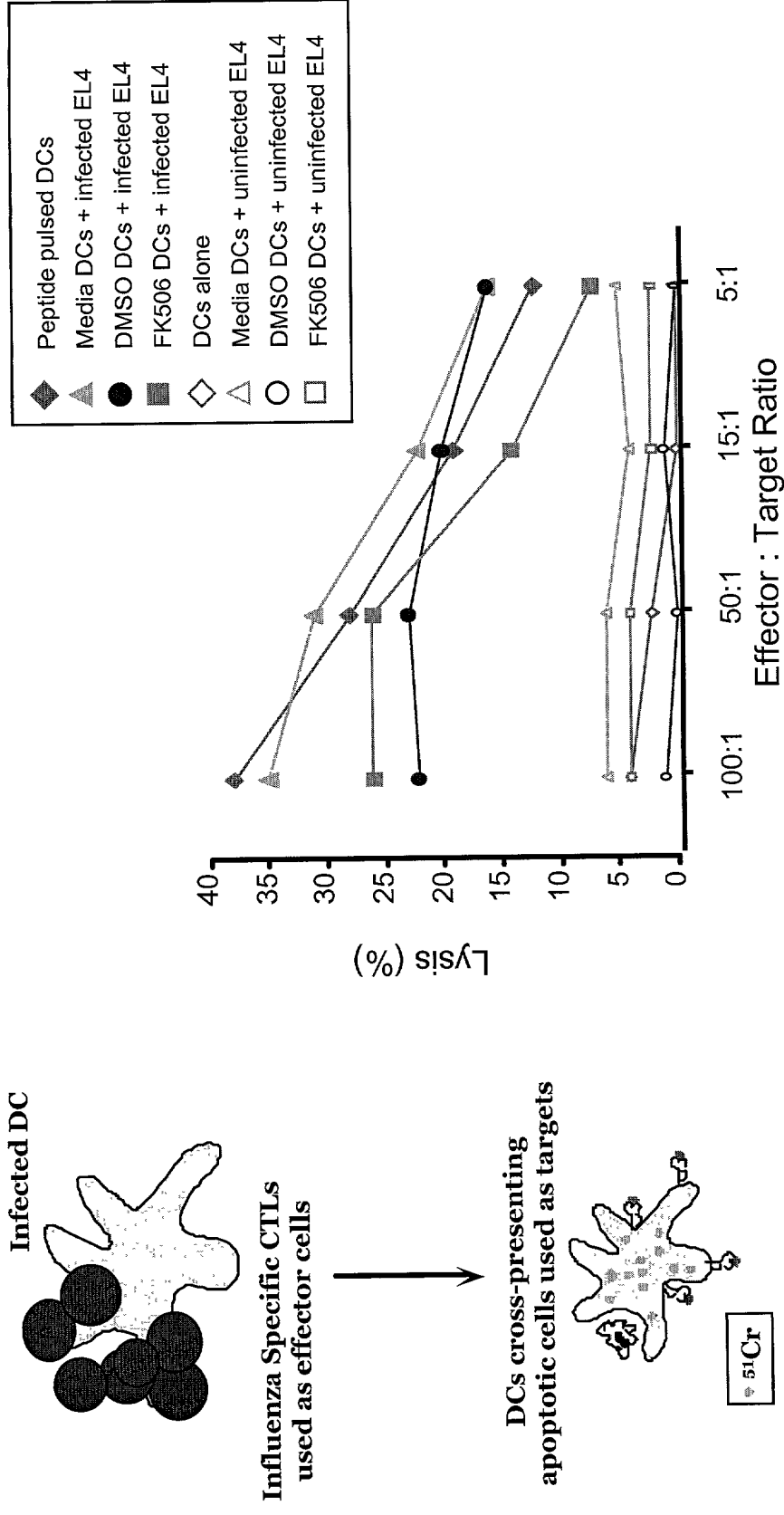
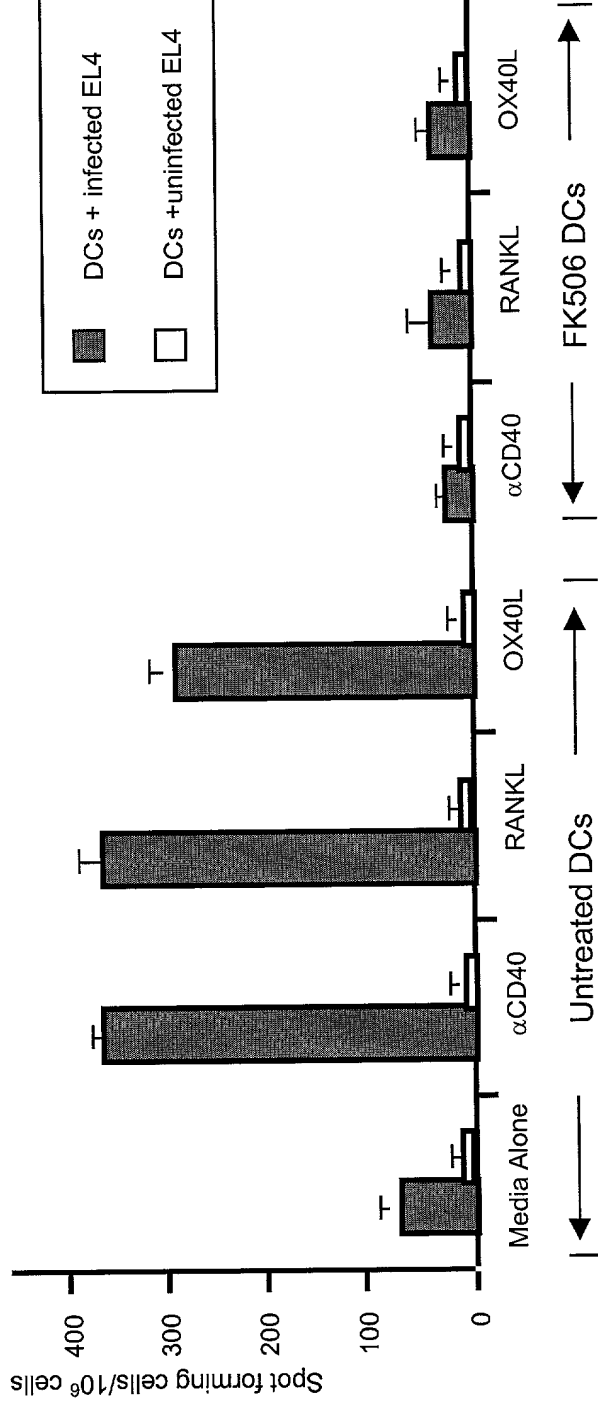


FIG 12

Cellular Immunology

FK506 acts to inhibit cross-priming by blocking signaling of TNF superfamily members



Cocultures were established as previously described +/- FK506 treatment. DCs were collected, counted and plated in wells containing purified CD8⁺ T cells with 1 μg/mL αCD40 antibody (Mabtech), human recombinant RANKL (Kamiya Biomedical), or human recombinant OX40L (Alexis Biochemicals). ELISPOT assay was performed and spot forming cells/10⁶ cells are reported. FK506 treated DCs block signaling of CD40, RANK and OX40 in the exogenous pathway.

IGNORANCE

IFN- γ

IFN- γ

Infected DCs

+

t=7 days

APCs + T cells

t=0 day

IFN- γ

IFN- γ

ACTIVATION

Apoptotic cells APC

Infected DCs

+

t=7 days

No IFN- γ producing T cells detected.

Demonstration of Cross-tolerization of influenza-specific T cells during 7 day coculture

IFN- γ producing cells indicates T cell ignorance. Neither activated at day 0 nor tolerized during 7 day culture.

Influenza-specific T cells

Deleted T cells

IFN- γ producing cells indicate T cell activation
Requirements Mature DCs & CD4 helper cells.

Fig 14

FK506 cross-tolerizes antigen specific CD8⁺ T cells

